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Influence of the cleaning system of conveyor belts on microbiological quality of poultry meat

[*Influência do sistema de autolimpeza das esteiras condutoras de cortes sobre a qualidade microbiológica da carne de frango*]

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ABSTRACT

This study focused on assessing the microbiology of conveyor belts surfaces, cleaned or not with pressurized water, and verification of the influence of this process on the microbiological quality of poultry meat. A reduction on mesophilic, psychrotrophic and *Enterobacteriaceae* counts (P<0.05) on dry conveyor belts compared to wet ones was observed. For the chicken leg (consisting of drumstick and thigh) samples, no statistically significant differences were detected on counts of indicators of microorganisms on poultry legs (composed by drumstick and thigh). For poultry meat conducted on wet or dry conveyor belts, 99% and 86%, were positive for *Listeria* spp, respectively. Only one sample of chicken leg was positive for presence of *L. monocytogenes*. These results demonstrate that there is no need to use water for cleaning conveyor belts during processing, which allows a reduction on the use of potable water in poultry slaughterhouses without jeopardizing food safety and public health.

Keywords**:** *Enterobacteriaceae, Listeria monocytogenes*, food microbiology, *Staphylococcus* spp.

RESUMO

Objetivou-se avaliar a qualidade microbiológica das superfícies das esteiras condutoras de cortes de carne de frango, higienizadas ou não com água pressurizada, bem como verificar a influência desse processo na qualidade microbiológica de cortes de frango. Foram observadas menores contagens, com diferença estatisticamente significativa (P<0,05) entre as populações de mesófilos, psicrotróficos e enterobactérias, nas esteiras condutoras de cortes secas em relação às úmidas. Nos cortes de coxa com sobrecoxa, as médias encontradas para populações de microrganismos indicadores não apresentaram diferenças estatisticamente significativas. Nos cortes analisados conduzidos pelas esteiras seca e úmida, 99% e 86% foram positivos para o isolamento de Listeria *spp., respectivamente. Apenas uma amostra de corte de coxa com sobrecoxa desossada foi positiva para a presença de* L. monocytogenes*. Os resultados demonstram a possibilidade do desligamento do sistema de autolimpeza das esteiras condutoras de cortes, obedecendo às questões de segurança dos alimentos e à saúde pública, o que resulta na redução do uso de água potável pelos matadouros-frigoríficos de aves.*

Palavras-chave: Enterobacteriaceae, Listeria monocytogenes, microbiologia de alimentos, *Staphylococcus* spp.

INTRODUCTION

The conveyor belts in cutting and deboning rooms in poultry slaughterhouses are used to allow hygienic conditions during meat processing. The sanitation procedure through water sprinkles on conveyor belts surfaces is routinely performed in slaughterhouses as required by the Ordinance No. 210 from Brazilian Ministry of Agriculture, Livestock and Food Supply (Brazil, 1998). The use of water during cleaning aims to prevent the occurrence

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of cross-contamination among meat cuts. However, water also spreads microorganisms in processing environment (Hamidi *et al*., 2014). According to Soares *et al.* (2014), the benefits and disadvantages of using water during poultry cuts transport on conveyor belts surface are not clear and a reduction on use of water during poultry meat processing is possible. However, its influence on the microbiological quality of poultry cuts was not evaluated. Thus, this study was performed in order to better understand the influence of using water during transport on conveyor belts on the microbiological quality of poultry cuts, in order to verify the risks for public health.

The indicator microorganisms are useful to evaluate the microbiological quality of food, providing information regarding food contamination degree and hygienic conditions during processing and storage (Santos, 2009). According to Silva *et al.* (2010), the total count of mesophilic aerobic bacteria is useful to obtain information about food shelf-life, the hygienic conditions of ingredients and the conditions of processing and handling. Another group used for evaluation of the hygienic condition of food is psychrotrophic microorganisms, which are responsible for food spoilage and modifications on sensory characteristics (Carvalho *et al.*, 2005).

Nevertheless, the main indicators of hygiene-sanitary conditions of food are bacteria included in *Enterobacteriaceae* family, such as *Escherichia coli* (Silva *et al.*, 2010), and *Staphylococcus* spp genus which includes species often associated with the hygienic conditions during food handling (Kwok e Chow, 2003). The presence of *Staphylococcus aureus* in food indicates that contamination occurred through improper handling or inadequate cleaning of utensils and equipment (Siqueira, 1995).

Furthermore, bacteria belonging to *Listeria* genus are also involved in food contamination and outbreaks (Franco e Landgraf, 2008). Among them, *Listeria monocytogenes* is considered as the most important pathogenic specie and its presence is reported at refrigerated environments (e.g. cutting rooms) and food due its ability to grown at low temperatures.

Considering these mentioned aspects, this study focused on assessing the microbiology of conveyor belts surfaces, cleaned or not with pressurized water, and verification of the influence of this process on the microbiological quality of poultry meat.

MATERIAL AND METHODS

This study was approved by the Research Ethic Committee (14.1.927.74.6) from Faculty of Animal Science and Food Engineering (FZEA) – University of São Paulo.

This study was performed during 2014 in an exporter slaughterhouse located in the state of São Paulo, Brazil with capacity to slaughter 160,000 chickens daily. The carcasses, after pre-cooling, are cut and deboned in "deboning room", an exclusive refrigerated room (12ºC) for this purpose. Thus, these carcasses are hung on hooks from an automatic cutting machine that cut carcasses as follow: breast meat, wings, sirloin and thighs with drumsticks. Afterwards, these cuts are transported on a polyethylene conveyor belt (12 meters) which moves clockwise and has a self-cleaning system that uses pressurized chlorinated water (0.5 to 2.0ppm) at room temperature during approximately one minute. This cleaning system of conveyor belts did not allow the direct contact of poultry cuts with the water. Mechanical cleaning and meat fragments removal were performed using stainless steel scrapers.

A total of 160 samples were collected during 10 distinct visits in the slaughterhouse. During each visit, a set of 16 samples were collected as follow:

Dry conveyors belts (4 samples per collection): swab of the beginning of conveyor belt (1 sample), poultry cut transported on the beginning of conveyor belt (1 sample), swab of the end of conveyor belt (1 sample), and poultry cut processed transported on the end of conveyor belt (1 sample)

Wet conveyors belts (4 samples per collection): swab of the beginning of conveyor belt (1 sample), poultry cut transported on the beginning of conveyor belt (1 sample), swab of the end of conveyor belt (1 sample), and poultry cut

processed transported on the end of conveyor belt (1 sample).

The sample collection was performed at two distinct times in the slaughterhouse: one hour before the first operational cleaning (1ºH) and the second operational cleaning (2ºH), totalizing 16 samples collected daily. The operational cleaning was performed in this industry when deboning was finished.

The samples of conveyor belt surfaces were collected using swabs in a area of 100cm² (established by sterile templates). Swabs were transferred to sterile Falcon tubes containing 10mL of 0.1% peptone water and others containing 10mL of LEB (*Listeria* enrichment broth, Difco Laboratories). All samples were collected during deboning operation. Two areas of 100cm² were sampled on the surface of conveyor belts - on its beginning and on the end of conveyor belts. The rinse technique on sterile plastic bags was used for chicken leg samples (Silva *et al*., 2010).

The content of falcon tubes were transferred to Erlenmeyer flasks containing 90mL of 0.1% peptone water (dilution 10^0) (Silva *et al.*, 2010). The samples of chicken cuts were weighed and 1mL of 0.1% peptone water was added for each gram of sample (dilution 10^0) After homogenization, 1ml of the initial dilution (10^0) was transferred to tubes containing 9ml of 0.1% peptone water (dilution 10^{-1}) to perform serial dilutions.

The mesophilic and psychrotrophic microorganisms were counted using Standard Plate Count Agar (SPC; Oxoid) (Compendium…., 2001). The incubation for counting mesophilic microorganisms was performed at 35ºC during 48 hours while for psychrotrophic microorganisms incubation was performed at 7°C for 10 days. The number of CFU was multiplied by the dilution factor in order to determine the CFU mL^{-1} or CFU g^1 in samples (.Compendium…, 2001). The sampled area was divided by the volume of diluent used and the results were expressed as CFU cm^{-2}.

For *Enterobacteriaceae* count (Compendium…, 2001), one mL of diluted samples was pipetted into sterile Petri dishes containing Violet Red Bile Glucose Agar (VRBG, Oxoid) which were incubated at 35°C during 18 to 24 hours. The number of CFU was multiplied by the dilution factor in order to determine the CFU mL^{-1} or CFU g^{-1} in the original sample (Compendium…, 2001). For *Staphylococcus* spp. count, 0.1mL was spread in a Petri dish containing Baird Parker Agar (BPA; Difco Laboratories) supplemented with egg yolk-potassium tellurite. After drying, these plates were incubated at 35°C during 48 hours (Compendium…, 2001).

Listeria spp. isolation was performed according to the methodology described by Donnelly *et al.* (1992). Aliquots of 25g of deboned chicken legs (thigh and drumstick) were added to 225mL of LEB. The samples were transferred to asterile polyethylene bag and processed in a stomacher for 1 minute. The swabs of surfaces were transferred to sterile Falcon tubes containing 10mL of LEB. The tube's contents were transferred to Erlenmeyer flasks containing 90mL of LEB and incubated at 30°C during 24 hours. After this period, an aliquot of 0.1mL was added to 10mL of Fraser broth (Difco Laboratories) supplemented with ferric ammonium citrate (Difco Laboratories) and then incubated at 35°C during 24 to 48 hours.

Then, the samples were sown using streaking technique on plates of Modified Oxford agar (MOX; Difco Laboratories) supplemented with colistin sulfate and moxalactam (Difco Laboratories) which were incubated at 35°C during 24 to 48 hours. The black, regular colonies, surrounded by dark halo (esculin hydrolysis) were considered as suspected for *Listeria* spp. Those characteristic colonies of *Listeria* spp. were stained by Gram staining. Five characteristic colonies of *Listeria* spp. were sown using the streaking technique on plates of Trypticase Soy agar supplemented with 0.6% yeast extract (TSYEA; Difco Laboratories). For those plates with count lower than five colonies, all colonies were selected. The plates were incubated at 30°C during 24 to 48 hours. The microorganisms were then inoculated into tubes containing Trypticase soy broth with yeast extract (TSYBE; Difco Laboratories) and incubated at 30°C during 24 hours. The biochemical characterization of *L. monocytogenes* was performed according to Silva *et al*. (2010).

The counts of microorganisms were converted to the base-10 logarithm of the number of colony-forming units plus 1. The statistical analysis of counts were performed through analysis of variance (ANOVA) and the means were compared using Tukey's test ($P<0.05$) according to Banzatto e Kronka, (2006). These data in transformed scale was evaluated by a general linear model procedure using PROC MIXED (SAS Institute).The adopted model is presented in the Explanatory Note. Data on *Listeria* spp. isolation were analyzed using a generalized linear model assuming binomial distribution with a logic link function and considering the different types of conveyor belts, and sample collection time and site. Analyses were performed using the PROC GENMOD procedure in the SAS statistical package version 9.1.3 (Statistical…, 2005). The model used for *Listeria* spp. is also presented in the Explanatory Note.

Explanatory Note

CFU

$Y_{ijkl} = \mu + E_i + H_j + L_k + EH_{ij} + EL_{ik} + HL_{jk} + EHL_{ijk} + e_{ijkl}$

Where, Y_{ijkl} = number of colony-forming units in transformed (repetition l, point k, time j, conveyor belt i); μ = constant inherent in all observations; E_i = effect of the ith conveyor belt, with i = 1 (dry) or 2 (wet); H_j = effect of the j-th sample collection time, with j = 1 (10:00) or 2 (18:00); L_k = effect of the k-th sample collection point, with k = 1 (beginning of the conveyor belt) or 2 (end of the conveyor belt); EH_{ij} = effect of double interaction existing between conveyor belt i*time j; EL_{ik} = effect of double interaction existing between conveyor belt i*point k; HL_{jk} = effect of double interaction existing between time j*point k; EHL_{ijk} = effect of triple interaction existing between conveyor belt i*time j*site k; e_{ijkl} = residual random effect associated with the number of colony-forming units in transformed scale (repetition l, site k, time j, conveyor belt i).

Listeria spp. (percentage)

$Y_{ijkl} = \eta(x) = \mu + E_i + H_j + L_k + EH_{ij} + EL_{ik} + HL_{jk} + EHL_{ijk} + e_{ijkl}$

Where, Y_{ijkl} = percentage of *Listeria* spp. detection (repetition l, sample collection site k, sample collection time j and conveyor belt i); η = logistic function that couples the binomial variable to the systematic component of the model; μ = constant inherent in all observations; E_i = effect of the ith conveyor belt, with i = 1 (dry) or 2 (wet); H_j = effect of the j-th sample collection time, with j = 1 (10:00) or 2 (18:00); L_k = effect of the k-th sample collection site, with k = 1 (beginning of the conveyor belt) or 2 (end of the conveyor belt); EH_{ij} = effect of double interaction existing between conveyor belt i*time j; EL_{ik} = effect of double interaction existing between conveyor belt i*site k; HL_{jk} = effect of double interaction existing between time j*site k; EHL_{ijk} = effect of triple interaction existing between conveyor belt i*time j*site k; e_{ijkl} = residual random effect associated with the percentage of *Listeria* spp. determination (repetition l, site k, time j, conveyor belt i).

RESULTS AND DISCUSSION

Table 1 presents the average counts of indicator microorganisms in samples collected at two different sites of the surface of conveyor belts (beginning and end).The populations of microorganisms were significantly higher ($P<0.05$) on wet conveyor belts compared to dry ones, except *Staphylococcus* spp., as shown in Table 1.

Table 1. Average populations of mesophilic and psychrotrophic bacteria, *Enterobacteriaceae* and *Staphylococcus* spp. in surface samples collected from wet or dry conveyor belts at two different times (1ºH and 2ºH) and two different areas of conveyor belts, in a poultry slaughterhouse

Microorganisms	Conveyor belts		Time of collection		Area of conveyor belts	
	Dry	Wet	1°H	2°H	Beginning	End
	CFU.cm^{-2}		CFU.cm^{-2}		CFU.cm^{-2}	
Mesophilic	3.4×10^{1b}	7.9×10^{1a}	5.4×10^{1a}	5.0×10^{1a}	3.8×10^{1a}	7.0×10^{1a}
Psychrotrophic	2.2×10^{1b}	4.6×10^{1a}	2.5×10^{1a}	3.9×10^{1a}	1.9×10^{1b}	5.2×10^{1a}
Enterobacteriaceae	4×10^{0b}	6×10^{0a}	4×10^{0a}	5×10^{0a}	4×10^{0a}	5×10^{0a}
Staphylococcus spp.	1.7×10^{1a}	1.1×10^{1a}	1.5×10^{1a}	1.3×10^{1a}	1.4×10^{1a}	1.4×10^{1a}

1°H= one hour before the first cleaning, 2°H= one hour before the second cleaning, CFU= colony-forming units. In the same line, different letters indicate significant differences through Tukey's test ($P<0.05$).

These results highlighted that the use of water probably influenced in the obtained results, not assuring an adequate cleaning of the conveyor belts surfaces. The fact that water spread microorganisms (Hamidi *et al.*, 2014) is worrying because psychrotrophic bacteria cause food spoilage and poultry meat had direct contact with the surface of conveyor belts previously to packaging.

Our results disagree with those reported by Soares *et al*. (2014). These authors analyzed the microbiology of the surface of conveyor belts submitted a continuous cleaning system that uses pressurized hot water (45°C) and did not detected statistically significant differences on the counts of mesophilic microorganisms and enterobacteria. However, the temperature of the water used in these experiments was not the same. These results demonstrate that the microbial contamination on the surface of conveyor belts can be higher when the water is used at room temperature. In a previous study, Bersot *et al*. (2012) suggested that this continuous cleaning with water is not necessary and its removal can reduce effluents discharge in reducing the environment. Furthermore, the water at every processing step can be a risk, requiring more attention to effective water management in the processing plan (Hamidi *et al*., 2014).

The population of psychrotrophic microorganisms on the end of the belts was statistically higher ($P<0.05$) than those samples collected on the beginning of conveyor belts. However, there was no difference on the other groups of microorganisms. Thus, the sample collection site (near or 12 meters from auto-cleaning system) did not influence the microbiological contamination of poultry cuts. The results of indicator microorganisms (mesophilic, psychrotrophic, *Enterobacteriaceae* and *Staphylococcus* spp.) on poultry meat are shown in Table 2.

Table 2. Average populations of mesophilic and psychrotrophic bacteria, *Enterobacteriaceae* and *Staphylococcus* spp. in samples of poultry meat on wet or dry conveyor belts at two different times (1ºH and 2ºH) and two different areas of the conveyor belts, in a poultry slaughterhouse

Microorganisms	Poultry cuts transported on conveyor belts		Time of collection		Area of conveyor belts	
	Dry	Wet	1°H	2°H	Beginning	End
	CFU.g^{-1}		CFU.g^{-1}		CFU.g^{-1}	
Mesophilic	9.5 x 10^{2a}	8.9 x 10^{2a}	9.7 x 10^{2a}	8.7 x 10^{2a}	9.4 x 10^{2a}	9 x 10^{2a}
Psychrotrophic	5.6 x 10^{2a}	7.4 x 10^{2a}	8.6 x 10^{2a}	4.8 x 10^{2b}	7.3 x 10^{2a}	5.7 x 10^{2a}
Enterobacteriaceae	1.0 x 10^{2a}	1.2 x 10^{2a}	1.1 x 10^{2a}	1.1 x 10^{2a}	1.1 x 10^{2a}	1.1 x 10^{2a}
Staphylococcus spp.	1.2 x 10^{2a}	1.1 x 10^{2a}	1.2 x 10^{2a}	1.1 x 10^{2a}	1.2 x 10^{2a}	1.1 x 10^{2a}

1°H= one hour before the first cleaning, 2°H= one hour before the second cleaning, CFU= colony-forming units. In the same line, different letters indicate significant differences through Tukey's Test ($P<0.05$).

The results of microbiological contamination thorough counting indicator microorganisms on poultry meat and areas of conveyor belts (beginning and end) were similar due to no detection of significant differences between chicken meat cuts conducted by dry or wet conveyor belts. Thus, the contact between poultry meat and the surfaces of conveyor belts (wet or dry) did not influence poultry meat contamination.

For samples collected at distinct times, only the populations of psychrotrophic microorganism were different. A higher prevalence of these microorganisms was observed at time 1ºH (first operational cleaning) compared to 2ºH (second operational cleaning) Although time 2ºH (second operational cleaning) represented a longer period of surfaces exposure, other factors could contribute to this difference, such as the training of food handlers regarding good manufacturing practices, the cleaning procedures of equipment that directly contact food and the chicken's size (weight) during evisceration procedures (this equipment need to be regulated according to each chicken's weight in order to reduce meat contamination with feces and bile).

The population of psychrotrophic microorganisms in the beginning and end of the

conveyor belts surfaces were higher on the end. However, this contamination did not influence the average count of psychrotrophic microorganisms on samples of poultry meat. No statistically significant difference was observed between both areas of the conveyor belts (beginning and end) where chicken legs samples were collected.

Table 3 shows the results of counts of *Listeria* spp. on conveyor belts surface (wet or dry) at different times (1ºH and 2ºH) and areas (beginning and end) and also on poultry meat cuts transported on them.

A higher prevalence (P<0.05) of *Listeria* spp. on the surface of dry conveyor belts was detected compared to wet conveyor belts. Under the conditions of this experiment, the use of no water on the conveyor belts provided a higher detection of this microorganism. These results could occurr due a reduction on the number of competitor flora because these microorganisms multiply under high-humidity condition. However, there was no significant difference on the counts of mesophilic and psychrotrophic microorganisms as shown in Table 1. On the other hand, *Listeria* spp. is more prevalent in refrigerated environments (i.e. cutting rooms) and food due its ability to multiply under low temperature and also on carcasses in which the competing microflora was reduced or eliminated through refrigeration in chillers (Ceruti, 2009). The presence of *Listeria* spp. in surfaces of conveyor belts could be related to biofilm formation on the equipment (McCarthy & Burkhardt III, 2012) because plastic conveyor belts allow a stronger *Listeria* spp. adhesion compared to stainless surface (Veluz *et al.*, 2012). Furthermore, surfaces roughness of conveyor belts is an important factor that affects cleaning and sanitizing (Chaturongkasumrit *et al.*, 2011).

Table 3. Prevalence of *Listeria* spp. in poultry meat (poultry legs) and surface of the dry or wet conveyor belts at two different times (1ºH and 2ºH) and two different sites (beginning and end), in a poultry slaughterhouse

Listeria spp.	Conveyor belts		Time of collection		Area of conveyor belts	
	Dry	Wet	1°H	2°H	Beginning	.End
Conveyor belt	57%[a]	32%[b]	44%[a]	44%[a]	42% [a]	47%[a]
Chicken legs	99%[a]	86%[a]	86%[a]	99%[a]	99% [a]	86%[a]

1°H= one hour before the first cleaning, 2°H= one hour before the second cleaning, CFU= colony-forming units. In the same line, different letters indicate significant differences through Tukey's test (P<0.05).

Santos *et al.* (2013) established a prevalence of 21.4% of *Listeria* spp. on the surface of equipment routinely used in poultry slaughterhouses, from which 66.7% were samples collected obtained on conveyor belts. These values are inferior to those obtained in the present study and probably this difference occurred due difference on sample size. *L. monocytogenes* was not isolated on the surface of conveyor belts, contradicting Chiarini (2007).

Listeria was isolated from 57% of the samples from dry conveyor belts and from 99% of chicken legs samples transported on belts surface. The prevalence on samples collected from wet conveyor belts was 32% while the prevalence on chicken meat cuts was 86%. The surface contaminated with *Listeria* spp. is a risk for pathogen transfer to food. Cross-contamination often occurs when equipment and utensils (such as cutting boards) that are used during food processing are not properly cleaned (Goh *et al.*, 2014). The control of *Listeria* spp. in processing facilities was considered as formidable task by Chiarini *et al.* (2009) due its occurrence even when proper hygiene and good manufacturing practices are adopted.

Regarding poultry cuts, no statistically significant differences were established, but the samples included in this study presented a high prevalence of *Listeria* spp. Only one sample of chicken leg was contaminated with *L. monocytogenes*. This sample was collected at the beginning of the wet conveyor belt surface before the first cleaning treatment (1ºH). Osaili *et al*. (2011) evaluated 280 samples of raw chicken and ready-to-eat chicken food and these

authors detected 76 samples contaminated with *Listeria* spp. and 15 with *L. monocytogenes*, demonstrating the potential risk for public health, which could be reduced through washing poultry legs with 5% potassium sorbate (González–Fandos & Dominguez, 2007). Besides the fact that only one sample was contaminated with *L. monocytogenes* and poultry meat is not commonly consumed raw or undercooked, the risk for public health exists due the possibility of cross contamination during homemade food processing though improper cleaning of utensils (Goh *et al.*, 2014).

CONCLUSION

The population of indicator microorganisms was higher in samples obtained on the surfaces of wet conveyor belts. At the same time, the populations of microorganisms on poultry meat transport on wet or dry conveyor belts did not differ, highlighting that the use of automatic cleaning system with water is not mandatory and its removal does not pose public health into risk and allow a reduction on the use of potable water in poultry slaughterhouses.

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